NUCLEAR SCIENCE AND TECHNIQUES **26**, 040302 (2015)

Efficient removal of uranium from mice by a novel compound of fullerence multi-macrocyclic polyamine derivatives*

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(Received July 28, 2014; accepted in revised form September 3, 2014; published online August 20, 2015)

Uranium removal efficacy of fullerence multi-macrocyclic polyamine derivatives (C_{60}-MMP), a novel chelating agent, was evaluated in mice. C_{60}-MMP was administrated intravenously into mice at 30 min after the uranium contamination. The molar ratio of chelating ligand/uranium was about 1 : 1. The results indicate that C_{60}-MMP can effectively prevent accumulation of uranium in liver at 8 h after C_{60}-MMP injection. At 48 h after the last injection, uranium deposition in liver of C_{60}-MMP treated mice is approximately 65% less than that of the control group. C_{60}-MMP reacted positively in promoting the removal of uranium from kidney, and the urinary uranium excretion increased significantly, compared with the control and DTPA-treated mice. However, repeated administration of C_{60}-MMP, and combined injection of DTPA and C_{60}-MMP, did not show desirable effects on uranium removal from mice. It implies that more investigations are needed for the treatment protocols and clinical applications of C_{60}-MMP.

Keywords: Uranium, Internal contamination, Removal, Fullerence multi-macrocyclic polyamine derivatives (C_{60}-MMP)

DOI: 10.13538/j.1001-8042/nst.26.040302

I. INTRODUCTION

As a long-lived and naturally occurring radioelement, uranium is widely used as nuclear fuel in fission reactors and weapons. It is also a hazardous heavy metal with serious biological/chemical toxicity and high radioactivity. Frequent accidental intake of uranium by workers or the general public may occur in its extraction and purification, industrial manufacture and use of uranium compounds, fabrication of nuclear fuels, etc., Such acute or chronic exposures can lead to internal contamination that may induce heavy-metal chemical and radiological toxicities [1, 2]. A large number of trials on animals and exposed human have shown that the major perniciousness of soluble uranium compounds *in vivo* is the chemical toxicity rather than the radiotoxic [3–5].

Although final conclusions cannot yet be drawn on cancer risks related to uranium internal exposure, the potential health hazards of uranium were recognized in early days of its application [6]. An increasing number of investigations of the pharmacology and toxicology of uranium compounds demonstrate that uranium is harmful to bone, kidney, liver, and central nervous system, and it perturbs the antioxidant defense system and other body systems of human beings [7–12]. The corresponding measure for uranium contamination largely depends on the intake pathways (inhalation, ingestion or wound), the level of exposure and treatment delay. For contamination by inhalation, blood chelation and lung washing are recommended. For contamination by ingestion, the proposed measures include gastric dressing, precipitation, purge and chelation of the absorbed fraction into blood. For uranium invades via wound, it is advised to adopt blood chelation, surgical excision and washing. Therefore, chelation therapy is the most universal and available treatment to alleviate the toxicity of uranium in any case [13, 14].

The ideal antidotes for uranium intoxication should form excretable uranium complexes of higher stability in tissues and body fluids, since the biological ligands will compete for uranium complexation under physiological conditions. To be applied *in vivo*, such antidotes should be of desired solubility and low toxicity at an effective dosage [15, 16]. Additionally, it is highly desirable that the chelating agent is orally available in long-term therapy. An effective chelating agent can reduce the uranium retention in bone surfaces and in soft tissues by the re-circulated uranium [16]. Considerable decorporation agents have been designed and evaluated in the chelation therapy of actinides [17–20]. The Radiation Laboratory of University of California at Berkeley has been designing improved actinide-sequestering agents for chelation therapy since early 1950's [12]. A library of Catecholamide (CAM), Terephthalamide (TAM) and Hydroxypyridinone (HOPO) ligands are investigated in decontamination of Pu(IV), Th(IV), Am(III), uranyl ion and Np(IV), etc. [10, 21]. Especially, two selected chelating agents, 3,4,3-LI(1,2-HOPO) and 5-LIO(Me-3,2-HOPO) are considered effective in decorporation of Pu(IV), Am(III), U(VI)O_2, Np(V)O_2, and much more valid than diethylenetriaminepentaacetate (DTPA), the clinical approved

* Supported by National Natural Science Foundation (Nos. 21071102, 91126013 and 21371124), Joint Funds of China National Natural Science Foundation and China Academy of Engineering Physics (No. U1330125) and National Fund of China for Fostering Talents in Basic Science (No. J1210004)
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chelator [1, 15, 22, 23]. Stradling and his co-workers carried out investigations on actinides decorporation for especially chelation of Pu(IV), Am(III), U(VI), Th(IV) with DTPA and 3,4,3-LIHOPO [24–27]. Domingo *et al.* studied on the removal efficacy of 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) in protecting against uranium toxicity *in vivo*, and the results demonstrated that Tiron was unrivalled in reducing uranium level in renal and bone. Unfortunately, this chelator failed to reduce skeletal uranium deposition in the case of delayed injection [8, 24, 28, 29]. Fukuda *et al.* investigated chelating efficacy of bicarbonate combined respectively with ethane-1-hydroxy-1,1-bisphoshonate (EHBP), deferiprone (L1) and other chelators in removing depleted uranium (DU) [30]. These chelating agents, however, can serve only as investigative drugs which are incompatible in the reduction of bone deposition, soft tissues burden and excretion of uranium.

It is reported that fullerence and its derivatives tend to distribute in kidney, liver and bone [31–33]. In this work, looking for effective uranium-sequestering agent, we synthesized several kinds of new compounds via introducing functional groups (such as cyclen and pyrocatechol) into fullerences. Among them, fullerence multi-macrocyclic polyamine derivative (C_{60}-MMP, $n = 2$, $W_m = 1887.53$; $n = 3$, $W_m = 2470.98$, Fig. 1) may well be a possible biomimetic uranium chelator based on preliminary test in toxicity, tissue biodistribution and blood clearance rate *in vivo*.

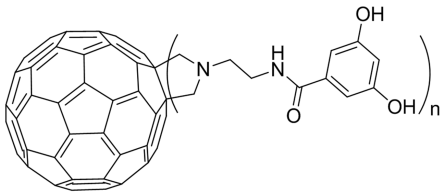

Fig. 1. Chemical structure of fullerence multi-macrocyclic polyamine derivatives (C_{60}-MMP).

In this work, C_{60}-MMP was further evaluated as a potential biomimetic uranium chelator. The uranium biodistribution and uranyl removal efficacy of C_{60}-MMP were investigated in uranium contaminated mice, and the chelating ligand potency was evaluated by comparing uranium retention and distribution in C_{60}-MMP-treated mouse groups with uranyl control and DTPA-treated groups. Regarding to the fact that the therapeutic effect may be affected by the pathway of exposure and chelation treatment protocol, comparative experiments were carried out between chronic low-dose and acute high-dose uranium poisoning mice, and between a single injection and repeated injections. In addition, the decorporation efficacy of using a combined drug (C_{60}-MMP and DTPA) in chelation therapy was evaluated by comparing with the efficacy of injecting C_{60}-MMP or DTPA alone.

II. MATERIALS AND METHODS

A. Chemicals and animals

DTPA, from Shanghai Chemical Reagent Supply Depot (Shanghai, China) was dissolved in 0.9% saline solution, adjusting pH to 7.4–8.4 with NaOH solution. Uranyl nitrate (Luxembourg, Germany) was prepared by dissolving uranyl nitrate hexahydrate in distilled water, and the solution of uranyl nitrate was adjusted to a pH 7 just before administered into mice. Since uranyl ion (UO_2^{2+}) is the most stable form in which uranium presents in biological body [17, 34, 35], uranium was administered as uranyl nitrate. Concentrations of the solution were adjusted to permit a dosing volume of 0.1 mL to inject.

C_{60}-MMP was synthesized in Department of Chemistry, Sichuan University (Chengdu, China). It was dissolved in 0.9% saline solution, adjusting the pH close to body fluid.

The experimental animals were 18–22 g adult Kunming mice (Huaxi Medical College, Sichuan University, Chengdu, China). They were used in accordance with guidelines on the care and use of laboratory animals. The 144 mice were housed in metabolic cages in groups, each of eight mice, after uranyl contamination. They were maintained on *ad libitum* diet and water, at relative humidity of 30%–70% and ambient temperature of 22–25 °C.

B. Determination of uranyl-removal efficacy

The mice were divided into 18 groups: six control groups (uranium contaminated only), six positive control groups (DTPA-treated groups) and six experimental groups (C_{60}-MMP-treated groups). Each mouse was given a dose of uranyl nitrate by lateral tail vein at a dosage of 0.1 mg. Positive control and experimental groups were respectively injected intravenously (i.v) with a single dose of DTPA and C_{60}-MMP 30 min after the contamination (i.v) of uranyl nitrate. The ligands were injected into mice at a ligand/uranium molar ratio of about 1 : 1 (about one-sixth of the intravenous LD_{50} value of DTPA and half of the intravenous LD_{50} value of C_{60}-MMP). Mice were humanitarian executed at 4, 8, 16, 24 and 48 h post-contaminated of uranium. The indicative tissues and organs, i.e. blood, liver, kidney and skeleton, were obtained. But the groups of 0.5 h were of blood-collection only. Urine and faeces were collected from the groups which sacrificed at 24 h and 48 h after the last injections to measure the faecal elimination of uranium.

C. Effect of repeated administration

Efficacy of repeated administration with low-dose chelating ligands on the degradation of uranium was measured by comparing the uranium biodistribution and faecal excretion in mice with those that were given only a single dose of high-dose chelator. Two groups of mice were given a single dose

of DTPA (0.33 μmol per mice) or C_{60}-MMP (0.33 μmol per mice) at 30 min post-contamination of uranyl nitrate. Another two groups of mice were administrated with three injections of DTPA and C_{60}-MMP (0.11 μmol per animal per dose), respectively. The injections were given at 0.5, 4 and 8 h after intravenous (i.v) contamination, respectively. The mice, if not given chelation therapy to collect urine and faeces, were kept in metabolic cage. Mice were sacrificed 24 h after the intravenous injection of UO_2^{2+}, and blood, liver, kidney, bone were removed from mice.

D. Efficacy of chelation therapy with combined drugs

Mice were divided into three groups. Group 1 was administrated with 0.17 μmol DTPA and 0.17 μmol C_{60}-MMP in sequence at 30 min after uranium contamination. Group 2 and Group 3 were treated with 0.33 μmol DTPA and C_{60}-MMP, respectively, after uranium injections. Urine and faeces were collected, and mice were sacrificed 24 h after the last injection, blood, liver, kidney and bone were removed from mice immediately.

E. Efficacy of chelation therapy in degradation chronic uranium toxicity

The 24 mice were contaminated with uranyl nitrate once a week for 4 weeks, and each mouse was given tail intravenous injection with a dosage of 0.08 μmol. Then mice were divided into three groups and kept separately at metabolic cage. The first group was given 0.1 mL of 0.9% saline, while the other two groups were treated with chelation therapy by administrating DTPA and C_{60}-MMP at a dose of 0.33 μmol per mice, respectively. To ensure the results reflecting the potential efficacy of the chelators at removing deposited U(VI) from target tissues like liver, kidney and bone, the chelating ligands were given at 48 h after the last injection of uranyl nitrate. At 24 h after the chelation therapy, mice were sacrificed and blood, liver, kidney and bone were collected.

F. Tissues processing and measurement

Wet samples were weighted just prior to pretreatment. Blood, livers, kidneys and bones were managed as individual samples, the separated urine and faeces were pooled for each group. Tissues and faeces were first dried at 200 °C and dry-ashed at 850 °C.Then the ashed samples were treated with wet digestion method (mixed with concentrated nitrate and hydrogen peroxide and heated under 200 °C). Urine samples were processed with concentrated nitrate and hydrogen peroxide directly. Uranium concentrations of the acquired sample were analyzed with WGJ-III laser-induced fluorescence (Hangzhou, China).

G. Data management and analysis

The final experimental data are expressed as uranium content of per gram of wet tissue weight (μg U/g b. wt.), in arithmetic means ± standard deviation. Each group was measured as a complete metabolic balance study. When comparing values between the groups, the term "significant" is used in the statistical sense, indicating $P < 0.05$ by one-way analysis of variance (ANOVA) followed by adequate post hoc analysis. The treatment groups were compared with controls by Student's t-test comparison test [13, 36].

III. RESULTS AND DISCUSSION

A. Effectiveness of C_{60}-MMP in uranium decorporation

Investigations on uranium decorporation efficacy of C_{60}-MMP were performed on experimental acute uranium poisoning mice. Chelating ligand potency was evaluated by comparing uranium retention in tissues and excretion in excreta of chelator-treated mice with the corresponding control groups, which were contaminated with uranyl nitrate only, and with the groups similarly treated with DTPA.

UO_2^{2+} shall be absorbed into the blood in a short time (10–30 min) before its distribution to target organs. If uranium is injected by tail intravenous, about two thirds of UO_2^{2+} will eliminated from plasma. Apart from the uranium being excreted by kidneys, approximately 20% of the total uranium will be deposited in the skeleton and 12% deposited in kidneys, and also liver is another mainly damaged organ [17, 37]. Take into account these facts and preliminary biodistribution results, blood, liver, kidney and skeleton were selected to assess the decorporation efficacy of the new chelating ligand. Biodistribution of uranium in blood and tissues was determined at different time intervals after the last injection. The data were used to analyze the metabolism of uranium *in vivo* and the changes of uranium concentration in organs and tissues after the chelation therapy.

Uranium concentration changes in blood (Fig. 2(a)) showed that UO_2^{2+} would strand in blood for a relatively longer time (8–16 h) in mice treated with C_{60}-MMP. The uranium concentration increased in the first eight hours, whereas uranyl ion concentrations in blood of untreated control mice and DTPA-treated mice reduced to background level in a short transition time. The increased uranium concentrations in blood of C_{60}-MMP-treated group in the first eight hours may contribute to the reduction of uranium concentration in kidney. Apart from excreting in the way of urine, uranyl ion will go partly into blood during the recirculation. Also, it indicates that uranium in blood will be distributed slowly into target organs, which is conducive for the antidotal efficacy of chelator. For the three groups, the blood uranium concentrations at 48 h are higher than those at 24 h, and the DTPA-treated (146%) and C_{60}-MMP-treated (∼86%) groups are significantly higher. This suggests that, comparing with the control group, a larger part of uranyl ions deposit in the target organs of treated mice may re-circulate in blood.

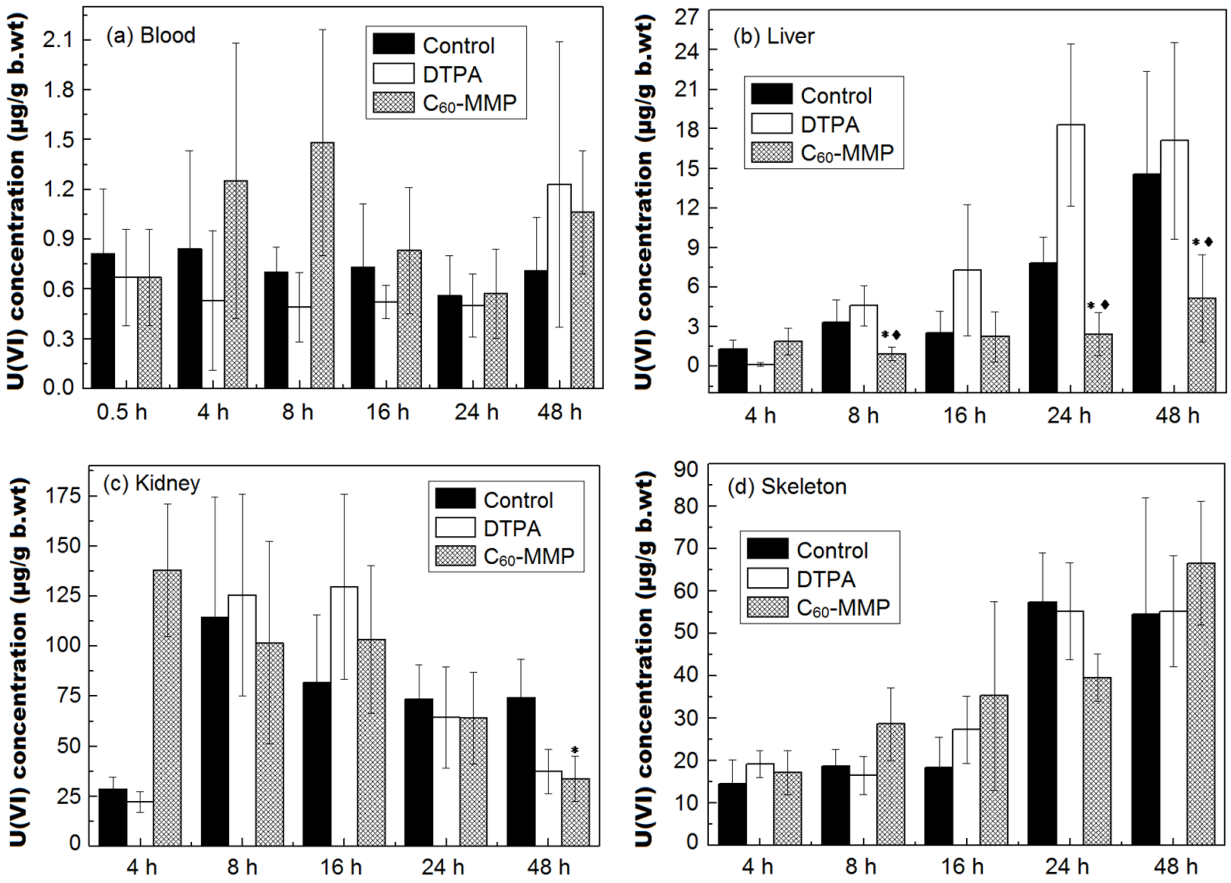

Fig. 2. U(VI) concentrations in blood (a), liver (b), kidney (c) and skeleton (d) after chelation therapy. The ligands were given to mice groups by IV injection at 30 min after IV injection of uranyl nitrate. The injection volumes were 0.1 mL. Data expressed as uranium concentration in unit wet mass of tissues or organs (μg/g b.wt, mean ± standard deviation). ♦, significantly less than the data from mice similarly treated with DTPA ($P < 0.05$, *t*-test); ∗, significantly less than the data from the U(VI) injected controls ($P < 0.05$, *t*-test).

Liver is another indicative sample of uranium toxicity, and uranium removal in liver is an important criteria of decorporation efficacy of the chelating ligand. The liver uranium metabolism in 48 h is shown in Fig. 2(b). Efficacy of C_{60}-MMP in decorporation of uranium in livers is quite obvious from 8 h post-injection of chelating ligand. At 48 h, averaged uranium content of livers in C_{60}-MMP-treated mice is 65% lower than the control group and nearly 70% lower than the DTPA-treated group. Together with the results of uranium concentration variations in blood, it is safe to conclude that the longer time span of uranium stay in blood will help to prevent metal ions from depositing in liver, hence the reduced difficulty of sequestering uranyl ions. With regard to the effect of DTPA in degradation of uranium poisoning in contaminated mice, the result is rather depressed. Compared with the U(VI) control groups, DTPA is not only ineffective in liver protection but also increases the retention of uranium, which is consistent with the experimental results of Durbin and Ortega *et al*. [8, 12, 16].

Figure 2(c) shows the uranium concentrations in kidneys at different hours after C_{60}-MMP injection. Uranium metabolism in kidneys of mice treated with C_{60}-MMP chelation therapy presents different tendencies. Concentrations of uranyl ion in kidney keep relatively high in 16 hours after injection. However, the high level of uranium concentrations in kidneys of U(VI) and DTPA-treated control groups only maintained in a relatively short time, ranging from 4 h to 8 h. Furthermore, significant reductions of uranium deposition in kidneys were observed in both chelation treated mice groups compared with the U(VI) control group. This indicates that most uranyl ions formed excretable compounds with chelators and expelled from body in the form of urine, since renal excretion is the major route of uranium elimination [8, 17].

Nevertheless, DTPA and C_{60}-MMP both failed to remove uranium that already deposited in bone (Fig. 2(d)). At 48 h after the chelation therapy, the U(VI) increase in skeleton of the C_{60}-MMP treated mice may contribute to understanding uranium recirculation in mice. It was reported that other chelating ligands, such as Gallic acid, EDTA and 3-LI(Me-3,2-HOPO), had similar results [8, 12].

A potential sequestering agent reduces uranium retention in organs and increases excreta elimination of uranium. Data of urinary excretion (Table 1) shows the therapeutic efficacy of chelator in prompting uranium excretion, and C_{60}-MMP is

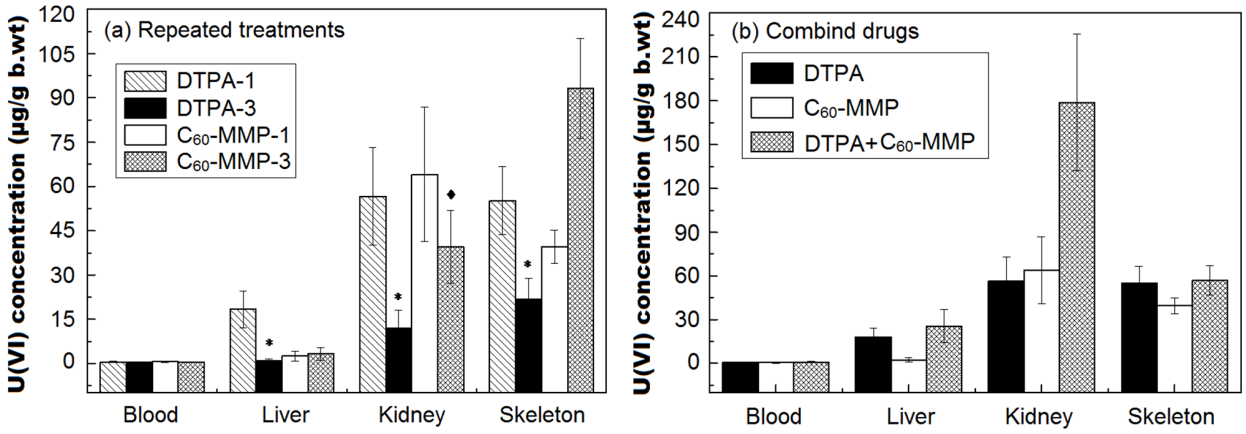

Fig. 3. U(VI) retention in mice treated with chelators repeatedly (a) and U(VI) distribution in mice treated with combined drugs (b).The ligands were given to the mice groups by IV injection at 30 min after IV injection of uranyl nitrate. The injection volumes were 0.1 mL. DTPA-3 and C_{60}-MMP-3 denote that the mice were treated respectively by DTPA (0.11 μmol/animal) and C_{60}-MMP, for three times. DTPA+C_{60}-MMP means that mice were treated with half dose (0.17 μmol/animal) of DTPA, and then with half dose of C_{60}-MMP. Data expressed as uranium concentration in unit wet mass of tissues or organs (mean ± standard deviation). ♦, significantly less than those of the mice similarly treated with DTPA ($P < 0.05$). ∗, significantly less than those of the U(VI) injected controls ($P < 0.05$).

TABLE 1. Excretion of uranium in mice after the chelation therapy with DTPA and C_{60}-MMP

Ligands[a]	Collection time (h)	Excretion of U(VI) (μg/animal), $n = 8$	
		Urine[b]	Feces[b]
Control	24	1.95	0.04
	48	1.61	0.16
DTPA	24	2.67	0.04
	48	2.48	0.16
C_{60}-MMP	24	4.33	0.21
	48	9.36	0.31

[a] Ligands were given to mice by IV injection of 0.1 mL uranyl nitrate.
[b] Excreta of each group were pooled and SD is not available.

much more effective than DTPA in increasing urinary excretion. We can see from Table 1 that urine, rather than feces, is the main route of uranium excreting from body, which was reported previously [16, 38].

The efficacy of C_{60}-MMP in uranium chelation may due to the fact that C_{60}-MMP has multiple uranium-binding units, and this uranium-binding unit, polyamine, could form rather stable complexes with uranyl ions [17, 39].

B. Effects of repeated chelator administration and combined drugs on chelation therapy of uranium toxicity

Good repeated low-dose validity is a desirable property of a chelating ligand, as a high-dose of chelator is toxic for human beings. Effectiveness of repeated treatment on poisoning mice with sequestering agents was evaluated, and results are shown in Fig. 3(a). Administrated in three times, with the same dosage of DTPA, uranium depositions in liver, kidney and skeleton are significantly less than the data from mice treated with a single injection. Repeated low-dose injection of C_{60}-MMP is of slight validity in reduction of renal uranium, but it markedly increases the retention of uranium in skeleton. Also, both DTPA and C_{60}-MMP are almost ineffective to promote the excretion of urinary uranium (Table 2). Together with the results of repeated administration of DTPA and C_{60}-MMP, we can have a preliminary conclusion that DTPA is effective in a low dosage yet C_{60}-MMP could only work in a relative high dosage.

TABLE 2. Excretion of uranium in mice after repeated administration of DTPA and C_{60}-MMP

Ligands[a]	Dosages (μmol/animal)	Excretion of U(VI) (μmol/animal), $n = 8$	
		Urine[b]	Feces[b]
DTPA	0.33	2.67	0.04
	0.11 × 3[c]	0.40	0.10
C_{60}-MMP	0.33	4.33	0.21
	0.11 × 3[c]	0.39	0.04

[a] Ligands were given to mice by IV injection of 0.1 mL uranyl nitrate.
[b] Excreta of each group were pooled and SD is not available.
[c] The 0.33 μmol/animal was administrated into mice in three injections, 0.11 μmol/animal per injection.

It is reported that the most promising approach to chelation therapy for U(VI) appears to be a combination of ligands with different decorporation performances to gain access to U(VI) in kidney and bone. Actually, there is a combination of effective low-toxicity ligands which takes advantage of the greater possibility of 5-LI(Me-3,2- HOPO) to chelate UO_2^{2+} in the kidneys and the greater potential of 5-LI-CAM(S) to chelate U(VI) that already deposited in bone [17]. In this study, eight mice were subsequently treated with DTPA and C_{60}-MMP after the injection of uranium nitrate. The results were compared with the mice treated with equimolar

amounts of either chelator alone. Figure 3(b) shows uranium distribution in blood and other target organs of mice which were given chelation therapy with combined ligands and DTPA or C_{60}-MMP alone. Unfortunately, the combination of DTPA and C_{60}-MMP was found to be invalid in elimination of uranium in all measured tissues and organs, compared with mice treated with DTPA and C_{60}-MMP alone. Instead, it significantly increased the retention of uranium in kidney. As shown in Table 3, there was little uranyl ions detected in urine and feces, because of the high uranium deposition in kidneys of mice that treated with combined drugs.

TABLE 3. Excretion of uranium in mice after the injection of combined drug

Ligands[a]	Excretion of U(VI) (μmol/animal), $n = 8$	
	Urine[b]	Feces[b]
DTPA	2.67	0.04
C_{60}-MMP	4.33	0.21
DTPA+ C_{60}-MMP[c]	0.03	0.02

[a] Ligands (0.1 mL) were given to mice by IV injection 30 min after IV injection of uranyl nitrate (0.1 mL).
[b] Excreta of each group were pooled and SD is not available.
[c] Mice were treated with 0.17 μmol/animal of DTPA, and then treated with 0.17 μmol/animal of C_{60}-MMP.

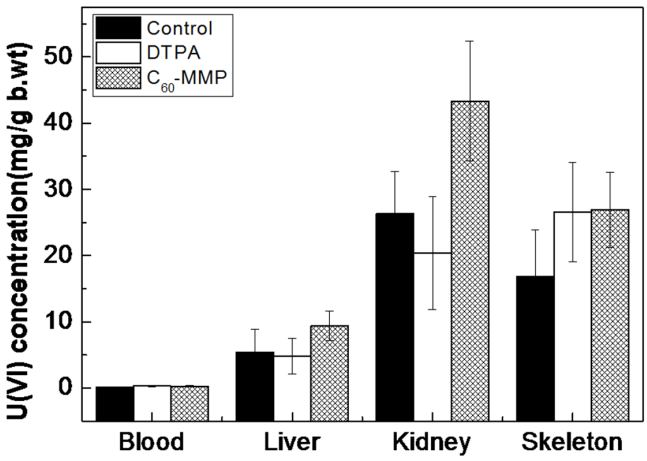

Fig. 4. U(VI) biodistribution in chronic poisoning mice after chelation therapy. Each mouse was contaminated with uranyl nitrate (0.17 μmol/animal) once a week for 4 weeks. Ligands (0.1 mL) were given to mice by IV injection 48 h after the last IV injection of uranyl nitrate. Data expressed as uranium concentration in unit wet mass of tissues or organs.

C. Efficacy of C_{60}-MMP in the treatment of uranium chronic toxicity

Prolonged exposure to low dosages of uranium can produce low level or "subclinical" illness and other detrimental effects [7], especially, chronic uranium poisoning is more common in the application of uranium. Therapeutic efficacy of C_{60}-MMP in the degradation of uranium chronic poisoning was evaluated in this work. It should be explained that excreta was not collected because of the fact about 60% of UO_2^{2+} excreted in the urine within 48 h.

Figure 4 shows the total uranium activities retained in the mice blood and organs. In general, chelators including DTPA and C_{60}-MMP were nearly ineffective in decorporation of uranium in liver, kidney and skeleton. Especially, uranium retentions in kidney and skeleton increased obviously in mice administrated with C_{60}-MMP. Therefore, it can be concluded that C_{60}-MMP is not suitable for the chelation therapy of uranium chronic poisoning.

IV. CONCLUSION

The first attempt has been made to evaluate the decorporation efficacy of C_{60}-MMP, a novel fullerence derivative, as a potential uranium removal ligand in mice. Experimental data supported that C_{60}-MMP could efficiently resist the uranium deposition in livers for the first 8 h and then increase the excretion of uranium in livers. In addition, C_{60}-MMP could help to speed up uranium metabolism and reduce its retention in kidneys. Urine was found to be the main pathway for uranyl elimination, of which C_{60}-MMP treated mice is much more significant than the other two groups of mice. Unfortunately, investigations on the effects of repeatedly administrated with low dose of C_{60}-MMP and combined with DTPA were almost noneffective in decrease of uranium deposition or uranyl urinary excretion. Moreover, C_{60}-MMP is not recommended using in the chronic uranium intoxications.

Although C_{60}-MMP is an efficient and novel uranium antidote, the present research represents only the first step in a systematic approach to the development of a rational therapeutic protocol for the treatment of uranium intoxications. More efforts should be made for improving treatment protocols and clinical applications. For the optimization of therapeutic efficacy, C_{60}-MMP is advised to combine with Tiron (Uranyl retention in bone is reported significantly reduced by Tiron in removing uranium in mice in the next survey [8]).

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